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Output Effect of Identified Ascending Interneurons upon the Abdominal Postural System in the Crayfish *Procambarus Clarkii* (Girard)

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ABSTRACT—The output effects of 16 identified ascending interneurons originating in the terminal abdominal ganglion were examined with intracellular recording and stimulating techniques in isolated nerve cord composed of 6 abdominal ganglia (from the 1st to 6th ganglion). The activity of the uropod closer and opener motor neurons was recorded extracellularly with pin electrodes from the terminal abdominal (A6) ganglion and that of the abdominal extensor and flexor motor neurons was recorded from the 1st to 5th abdominal (A1-A5) ganglion. This technique allowed us to monitor the activity of the motor neurons innervating different muscles of more than 10 simultaneously in the same preparation. Majority of ascending interneurons had output effects upon not only the uropod motor neurons but also the abdominal postural motor neurons. The premotor effects of the ascending interneurons were the same in all of abdominal ganglia (A1-A5). Some ascending interneurons also affected the abdominal postural motor neurons on both sides of each ganglion with a similar fashion. Neurobiotin staining revealed that the ascending axons spreaded their branches in each abdominal ganglion. Their branches were extended within the side ipsilateral to their axons. The possible function of ascending interneurons as multi-functional units in the sensory-motor system of crayfish was discussed. Since they received sensory inputs from the tailfan and affected the activity of both uropod and abdominal postural motor neurons simultaneously, they would coordinate the behavioural sequence controlling both the uropod motor system and abdominal postural system.

INTRODUCTION

The central nervous system (CNS) of arthropod animals, like insects and crustacean, consists of a series of segmental ganglion chained by a pair of connectives [e.g. 1]. Each ganglion is bilaterally symmetrical and contains most of motor neurons for its relevant segment. A segmental movement is basically controlled by local circuit within its relevant ganglion [2], then a series of movement and postural changes must be coordinated by intra- and intersegmental activation of different muscles [16, 31, 32].

For example, crayfish avoidance reaction consists of serially ordered behavioural acts [26]. Unilateral mechanical stimulation of the tailfan elicited a rapid closing movement of uropods followed by the completion of locomotor acts with a change in abdominal posture and pattern generation of walking legs. This assembly of elementary acts must be activated sequentially at the level of central neurons, since few afferents projected anteriorly through the abdominal nerve cord [11]. Neural elements controlling uropod motor system and abdominal postural system have been so far analyzed respectively. Motor neurons in each system have been identified [24, 36 in uropod motor neurons; 8, 17, 40 in abdominal postural motor neurons]. A vast number of premotor interneurons which affected the activity of motor neurons has been also characterized both physiologically and morphologically [20–22, 25 in uropod system; 13,18, 19, 37 in abdominal postural system]. Many of earlier behavioural and physiological works have expected that certain interneurons would contribute to the segmental linking between uropod and abdominal movement, though no attempt has been carried out to clarify this point.

About 65 pairs of ascending interneurons originating in the terminal abdominal ganglion of the crayfish [10, 33] have ascending axons through the anterior abdominal connective and receive sensory inputs directly from the tailfan [23, 34]. Twenty-four ascending interneurons are identified as unique individuals, and many of them affect the activity of the antagonistic sets of uropod motor neurons [21]. These identified ascending interneurons should be, therefore, the most possible candidate to mediate intersegmental coordination between uropod and abdominal movement. The present study examined this hypothesis to characterize their output effects upon the abdominal postural motor neurons.

Our results show the majority of ascending interneurons have premotor effects upon the abdominal postural motor neurons from the 1st to 5th abdominal ganglion as well as upon the uropod motor neurons. The homologous postural motor neurons throughout the anterior abdominal ganglia are affected in a similar fashion by ascending interneurons.

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MATERIALS AND METHODS

Animals and preparations

Adult male and female crayfish, *Procambarus clarkii* (Girard) (5–9 cm body length from rostrum to telson) were used in all experiments. They were obtained commercially and maintained in laboratory tanks before use.

Abdominal nerve chain including all abdominal (A1-A6) ganglia with relevant nerve roots was isolated from the abdomen. This preparation was pinned, ventral side up, to the floor of a Sylgard-lined Petri dish and perfused continuously with cooled physiological saline [38].

Extracellular recording and stimulation

The activity of motor neurons innervating closer and opener muscles of exopodite was recorded extracellularly by using pin electrodes from the 2nd and 3rd motor root in the terminal (6th) abdominal ganglion respectively [25]. In the anterior abdominal ganglia (from the 1st to 5th ganglion), the activity of tonic extensor motor neurons was recorded from the 2nd root and the activity of tonic flexor motor neurons was recorded from the superficial 3rd root of each ganglion. The pin electrodes were placed contact with each nerve root and insulated with petroleum jelly (Vaseline : liquid paraffin=3 : 1 in the volume) to perform multi-recording from a single preparation (Fig. 1).

Fig. 1. The picture of experimental arrangement with extracellular and intracellular electrodes. The abdominal nerve cord was dissected out from the 1st to terminal abdominal (A6) ganglion. The motor activities of the uropod and abdominal postural system were monitored from relevant motor nerve root with pin electrodes. Electrical stimulation was delivered to the 2nd root afferents of the terminal abdominal ganglion by the bipolar electrode. The intracellular recording and stimulation was made from the terminal abdominal ganglion.

Each motor neuron of the tonic abdominal postural system was identified by its spike amplitude and firing pattern of the extracellular recordings [4, 7, 8, 40]. Six tonic extensor motor neurons (EX) and 6 tonic flexor motor neurons (FL) were numbered in order of increasing spike size (i. e. No. 1 is the smallest and No. 6 is the largest). The second largest spike in each extensor or flexor motor neuron was the peripheral inhibitor, No. 5. In *in vitro* preparation, the peripheral inhibitor of the extensor motor neurons (No. 5) showed tonic discharge, although the largest unit of the extensor excitors (No. 6) did not fire spontaneously [37]. On both of the extensor and flexor excitor units, No. 3 and 4 and No. 1 and 2 were similar in amplitude within each grouping and thus were distinguishable as single unit, i. e., there were two No. 3/4 and two No. 1/2 spikes [15]. The premotor effect of ascending interneurons upon the abdominal postural motor neurons was judged to be excitatory if depolarizing current injected into the interneuron elicited or increased tonic spikes of excitors (No. 1/2, No. 3/4, and No. 6) and/or decreased tonic spikes of the inhibitor (No. 5). Inhibitory interaction was identified by the decrease in tonic spikes of excitors and/or the increase in tonic spikes of inhibitor. When the current injection caused no change in activity of motor neurons, the ascending interneuron was judged to have no obvious output effect.

The mechanosensory afferents (2nd root) innervating the right exopodite [3] were stimulated electrically by the bipolar stimulating pin electrode. Square pulses of 0.1 ms duration at 20 Hz were delivered to the stimulating electrode. Stimulus intensity was determined to be appeared that spikes of closer motor neuron increased and spikes of opener motor neurons decreased.

Intracellular recording and staining

Intracellular recordings were made with glass microelectrodes filled either with a 3% solution of Lucifer yellow CH [35] with 0.1 M lithium chloride (100 to 150 MΩ resistance) or a 3% solution of neurobiotin [9] with 1 M potassium chloride (40 to 80 MΩ resistance). Interneurons were always impaled in the right half of the neuropiler processes or axons in the terminal abdominal ganglion. A constant polarizing current could be injected into interneurons through the recording electrode by a bridge circuit to characterize their premotor effects upon both the uropod and abdominal postural motor neurons. In most records, the monitor of membrane potential of ascending interneurons during current injection, especially that of depolarization, was difficult because of extremely high resistance, so only the monitor of current was displayed.

After physiological examination, Lucifer yellow was injected into the interneuron with hyperpolarizing current pulses of 10 nA of 500 msec in duration at 1 Hz for 20 min. The ganglion was then fixed in a 10% formalin for 20 min, dehydrated with an alcohol series and cleared with methyl salicylate. Ascending interneurons were observed using a fluorescence microscope in whole mount, and photographed for subsequent reconstruction.

Neurobiotin was injected into the interneuron with depolarizing current pulse of 10 nA of 500 msec duration at 1 Hz for 3 hr. The preparations were then diffused for 10 hr at room temperature and fixed in a 10% formalin over 1 hr. They were dehydrated and immersed in methyl salicylate for 30 min to increase staining intensity and reduce background staining. They were rehydrated with an alcohol down series, rinsed with detergent A: 0.01% Triton X-100 and 0.01% Tween 20 in 0.15 M sodium phosphate buffer solution (pH 7.4) (PBS), and immersed in detergent B (2% Triton X-100 and 2% Tween 20 in PBS) for 90 min. After rinsed with detergent A 3 times for 5 min each, they were incubated in HRP conjugated streptavidin for 30 min. They were rinsed with detergent A 3 times for 15 min each, immersed in 0.025% diaminobenzidine (DAB) in PBS for 20 min and reacted with DAB and H_2O_2 (0.003%) in PBS.

After rinsed with DW several times, they were dehydrated and cleared. Ventral up views of the dye-filled cells were drawn with the aid of a camera lucid.

Each ascending interneuron in this study was identified as the criteria described previously [21]. According to their gross morphology (including soma position, number of main branches and axonal projection in the connective) and physiological properties (input from sensory afferents and output to uropod, closer and opener motor neurons), 24 ascending interneurons were divided into 6 classes; co-activating (CA), co-inhibiting (CI), reciprocally closing (RC), reciprocally opening (RO), no effective (NE) and variably effective (VE) interneurons. In total, 103 crayfish were studied in this paper and 78 ascending interneurons were identified and analyzed their output effect upon abdominal postural motor neurons. Other types of ascending interneurons were encountered only once and were not described here.

All the recordings were stored on a digital tape recorder (Biologic DTR-1801) and displayed using a chart recorder (Gould TA240S).

RESULTS

Serially ordered motor pattern elicited by stimulation of the uropod

Mechanical stimulation of the exopodite in small crayfish (less than 8 cm in length from rostrum to telson) frequently produced avoidance "dart" response ($P<0.0001$ with Chi square test) (Fig. 2A). The animals showed bilateral closing of uropods and forward walking with abdominal extension.

Repetitive electrical stimulation of the 2nd root afferents which innervated hairs on the surface of the exopodite increased the spike frequency of the closer motor neurons and decreased that of the opener motor neurons on the

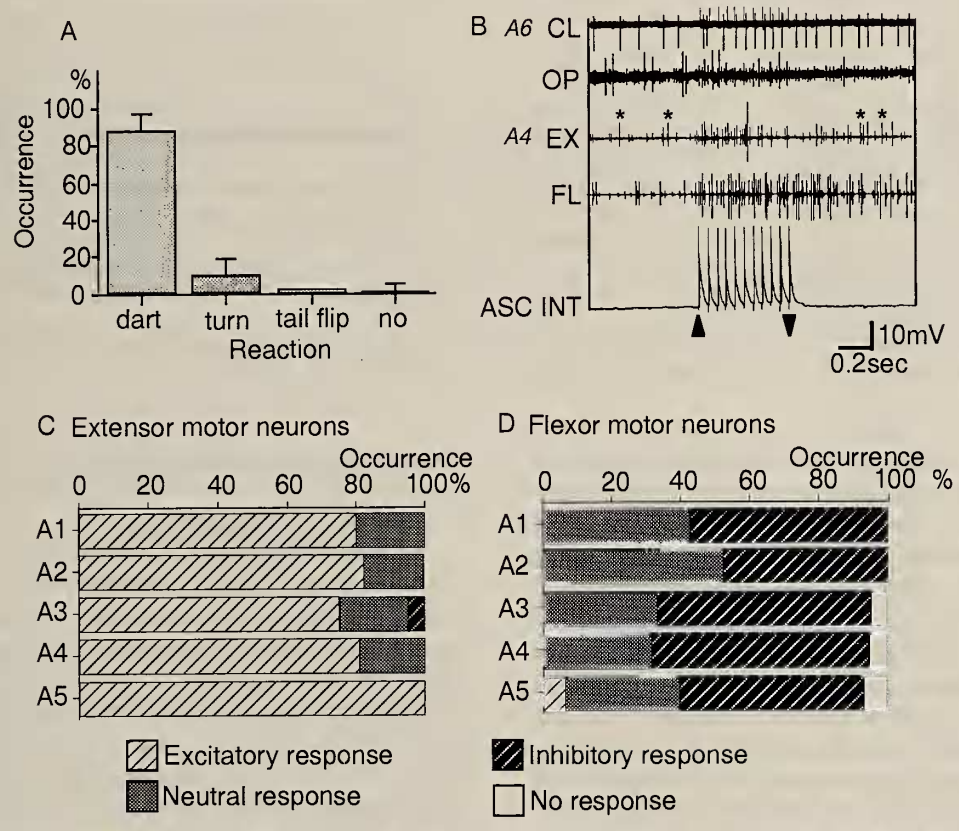

FIG. 2. Effect of sensory inputs upon the abdominal postural motor neurons. A. Patterns of crayfish reactions in response to unilateral mechanical stimulation of the exopodite. Twenty-five animals were used in the test and 20 trials were made in each. Observed reactions of the animals were categorized as one of four types: dart, turn, tailflip and no response [26]. B. Typical pattern of spike activity of motor and interneurons in response to the repetitive electrical stimulation (20 Hz) of the 2nd root afferent innervating the exopodite (indicated by arrowheads). The 1st and 2nd traces were extracellular recordings from the uropod, closer and opener motor neurons in the terminal abdominal ganglion. The 3rd and 4th traces were from the extensor and flexor motor neurons in the 4th abdominal ganglion. The spike unit indicated by asterisks in the 3rd trace was tonic extensor inhibitor (No. 5). The 5th trace was intracellular recording of the ascending interneuron (ASC INT) from the terminal ganglion. C and D. Pattern of the activity of the abdominal postural motor neurons (C: tonic extensor motor neurons, D: tonic flexor motor neurons) from the 1st to 5th abdominal ganglion (A1-A5) in response to electrical stimulation of the 2nd root afferents. The results were based on 21 preparations. The pattern of motor response was judged to be excitatory if stimulation of the afferents elicited or increased the spikes of excitors and/or decreased the spikes of the inhibitor. Inhibitory response was identified by the decrease in the spikes of excitors and/or the increase in the spikes of inhibitor. Neutral response was defined as when both the excitatory and inhibitory motor neurons were excited simultaneously.

Table 1. Summary of output effects of the identified ascending interneurons upon the abdominal postual motor neurons and uropod motor neurons

ASC INT	n	A1		A2		A3		A4		A5		A6		INPUT
		EX	FL	EX	FL	EX	FL	EX	FL	EX	FL	CL	OP	
CA-1	3	E	E	E	E	E	E	E	E	?	?	E	E	EPSP
CI-1	3	E	E	E	E	E	E	E	E	E	E	I	I	EPSP
RC-2	6	E	I	E	I	E	I	E	I	E	I	E	I	EPSP
RC-3	3	E	E	E	E	E	E	E	E	E	E	E	I	EPSP
RC-4	3	I	E	I	E	I	E	I	E	I	E	E	I	EPSP
RC-6	6	E	E	E	E	E	E	E	E	E	E	E	I	EPSP
RC-7	2	E	I	E	I	E	I	E	I	E	I	E	I	EPSP
RC-8	7	E	E	E	E	E	E	E	E	E	E	E	I	EPSP
RO-2	2	I	E	I	E	I	E	I	E	I	E	I	E	EPSP*
RO-3	2	I	E	I	E	I	E	I	E	I	E	I	E	EPSP*
RO-4	5	I	E	I	E	I	E	I	E	I	E	I	E	IPSP
RO-5	2	E	E	E	E	E	E	E	E	E	E	I	E	EPSP
RO-6	5	E	I	E	I	E	I	E	I	E	I	I	E	EPSP
VE-1	19	E	I	E	I	E	I	E	I	E	I	V	V	EPSP
NE-1	7	E	E	E	E	E	E	E	E	E	E	N	N	EPSP
NE-2	3	N	N	N	N	N	N	N	N	N	N	N	N	EPSP

n: Sampling number of ascending interneurons E: Excitatory effect; output effect upon the abdominal postural motor neurons was judged to be excitatory if current injection elicited or increased the spikes of excitors and/or decreased the spikes of inhibitor. I: Inhibitory effect; inhibitory effect was identified by the decrease in the spikes of excitors and/or the increase in the spikes of inhibitor. V: Variable effect; when the current injection caused inconstant change in the activity of motor neurons, the ascending interneuron was judged to be variable effect. N: No effect; when the current injection caused no change in the activity of motor neurons, the ascending interneuron was judged to be no effect. ?: No record *: Interneurons showed antifacilitation when repetitive electrical stimulation (20 Hz) was applied.
Input of each interneuron was characterized by the electrical stimulation of the 2nd root afferents of terminal abdominal ganglion on the side ipsilateral to axons of interneurons.

stimulating side (Fig. 2B). At the same time, the activity of the abdominal postural motoneurons was also changed by the electrical stimulation. On the extensor motor neurons from the 1st to 5th abdominal ganglion, inhibitory motor neuron (No. 5) decreased the spike frequency and excitatory motor neurons increased the spike frequency ($P<0.0001$ with Chi square test) (Fig. 2C). The response of the flexor motor neurons was somewhat variable, but the spike frequency of the flexor inhibitor (No. 5) was usually increased (Fig. 2D). Thus, mechanosensory stimulation elicited abdominal extension-like motor pattern in the anterior abdominal ganglia as well as closing pattern of the uropod in the terminal abdominal ganglion.

Output effects of ascending interneurons upon abdominal postural motor neurons

Since the majority of branches in the sensory afferents ended in the terminal abdominal (A6) ganglion [11], the change in the activity of the abdominal postural motor neurons induced by sensory stimulation (Fig. 2C and D) was mediated by certain interneurons with intersegmental projection. Ascending interneurons originating in the terminal abdominal ganglion had intersegmental ascending axon and received sensory inputs directly from the afferents [21]. In this study, 16 identified ascending interneurons were characterized by their premotor effects upon both the extensor and flexor motor neurons from the 1st to 5th abdominal (A1-A5) ganglion (Table 1).

Of 16 identified ascending interneurons, 15 interneurons had output effect upon the abdominal postural motor neurons. Seven interneurons excited both the extensor and flexor motor neurons co-actively. Four interneurons had excitatory effects upon the extensor motor neurons and inhibitory effects upon the antagonistic flexor motor neurons. Another four interneurons had excitatory effects upon the flexor motor neurons and inhibitory effects upon the extensor motor neurons. One identified interneuron (NE-2) had no obvious effect upon the abdominal postural motor neurons.

Interneurons with co-activating effect

Ascending interneurons identified as CA-1, CI-1, RC-3, RC-6, RC-8, RO-5 and NE-1 [21] activated both the extensor and flexor motor neurons (Table 1).

For example, CA-1 was identified by its soma location of rostralateral region and two main neurites projected laterally (Fig. 3A). Physiologically, this interneuron excited both the closer and opener motor neurons (not shown). In the 1st abdominal ganglion, tonic spikes of extensor inhibitor (No. 5) were inhibited by the passage of depolarizing current (top in Fig. 3B). At the same time, this interneuron increased the

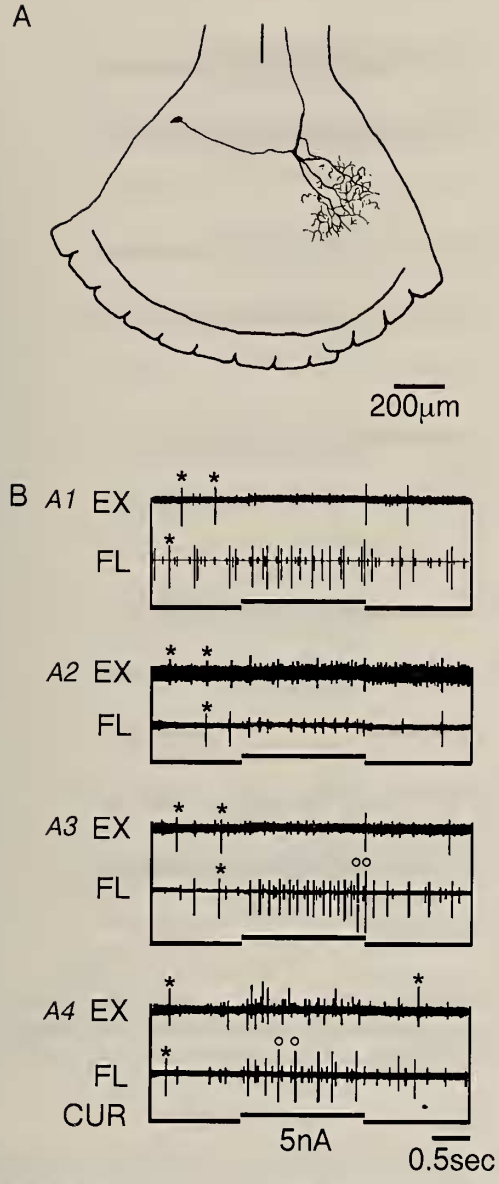

FIG. 3. Output effect of CA-1. A. Morphology of CA-1 in the terminal ganglion. Interneuron was drawn within the outline of the ganglion to show its relative position in the ganglion. B. Output effect of CA-1 upon both the extensor (EX) and flexor (FL) motor neurons in the abdominal (A1-A4) ganglia. In this paper, the spike unit indicated by asterisks was tonic extensor or flexor inhibitor (No. 5) and circles was the largest spike unit of excitor (No. 6).

spike frequency of the flexor excitors (No. 1/2 and 3/4). In the 2nd abdominal ganglion, this interneuron increased the spike frequency of the extensor excitors (No. 3/4). At the same time, the flexor excitors spiked and tonic spikes of the flexor inhibitor were suppressed during current injection. In the 3rd abdominal ganglion, tonic spikes of extensor inhibitor (No. 5) were inhibited while those of flexor excitors (No. 1/2 and 3/4) were increased. In the 4th abdominal ganglion, this interneuron increased tonic spikes of excitors of both the extensor and flexor motor neurons. Thus, CA-1 excited the excitors of both the extensor and flexor motor neurons and inhibited the extensor inhibitors of from the 1st to 4th abdominal ganglion, though we could not test the effect of the interneuron upon the 5th abdominal ganglion (Fig. 3B).

NE-1 was characterized by its thick axon and the limited extent of main branches (Fig. 4A). Neurobiotin staining showed that the ascending axon ran through, at least, the 4th abdominal ganglion. In the 5th abdominal ganglion, several small branches projected mainly medially from the axon. No axonal branches, however, crossed the midline. Physiologically, NE-1 had no obvious output to the uropod motor neurons. The spike frequency of either closer or opener motor neurons did not change significantly even if depolarizing current of high intensity was injected into the interneuron (12 nA in Fig. 4B). This interneuron, however, affected the abdominal postural motor neurons in the anterior abdominal ganglia. In each abdominal ganglion (A1-A5), the passage of depolarizing current increased the spike activity of both the extensor and flexor excitors (Fig. 4C). Thus, NE-1 had output effect upon the abdominal postural motor neurons, though it had no effect upon the uropod motor neurons. NE-2 (not shown) was another identifiable ascending interneuron which had no output effect upon the uropod motor neurons [21]. In this study, NE-2 was obtained three times but no output effect upon the abdominal postural motor neurons was recognized.

The other interneurons, CI-2, RC-3, RC-6, RC-8 and RO-5 had similar co-activating effect upon the antagonistic extensor and flexor motor neurons, though their effects upon the uropod motor neurons were variable (Table 1).

Interneurons with extension effect

Four identified interneurons, RC-2, RC-7, RO-6, and VE-1 [21], elicited abdominal extension-like motor pattern. For example, RC-2 was characterized by its looped primary neurite from the soma (A6 in Fig. 5A) and the output effect of reciprocally closing pattern upon the uropod motor neurons (Fig. 5B). This interneuron could produce closing movement of exopodite. Neurobiotin staining revealed the morphology of ascending axon through the 3rd abdominal ganglion (Fig. 5A). Many small branches extended both medially and laterally within the axon side of the interneuron in each anterior ganglion. When the depolarizing current was injected into RC-2, this interneuron increased the spike frequency of the flexor inhibitor (No. 5) in all anterior abdominal ganglia (A1-A5) with the inhibition of the spikes in the flexor excitors (Fig. 5C). At the same time, this interneuron significantly increased the spike frequency of the extensor excitors, especially those in the posterior ganglia (A4 and A5). In the 1st abdominal ganglion, this interneuron decreased the frequency of small extracellular spikes (No. 1/2) in the extensor excitors but increased that of intermediate spikes (No. 3/4) (top in Fig. 5C). RC-7 had similar output effect to RC-2 upon both the uropod and abdominal postural motor neurons, while RO-6 had reversed effect upon the uropod motor neurons (Table 1).

VE-1 was identified by its characteristic shape of the

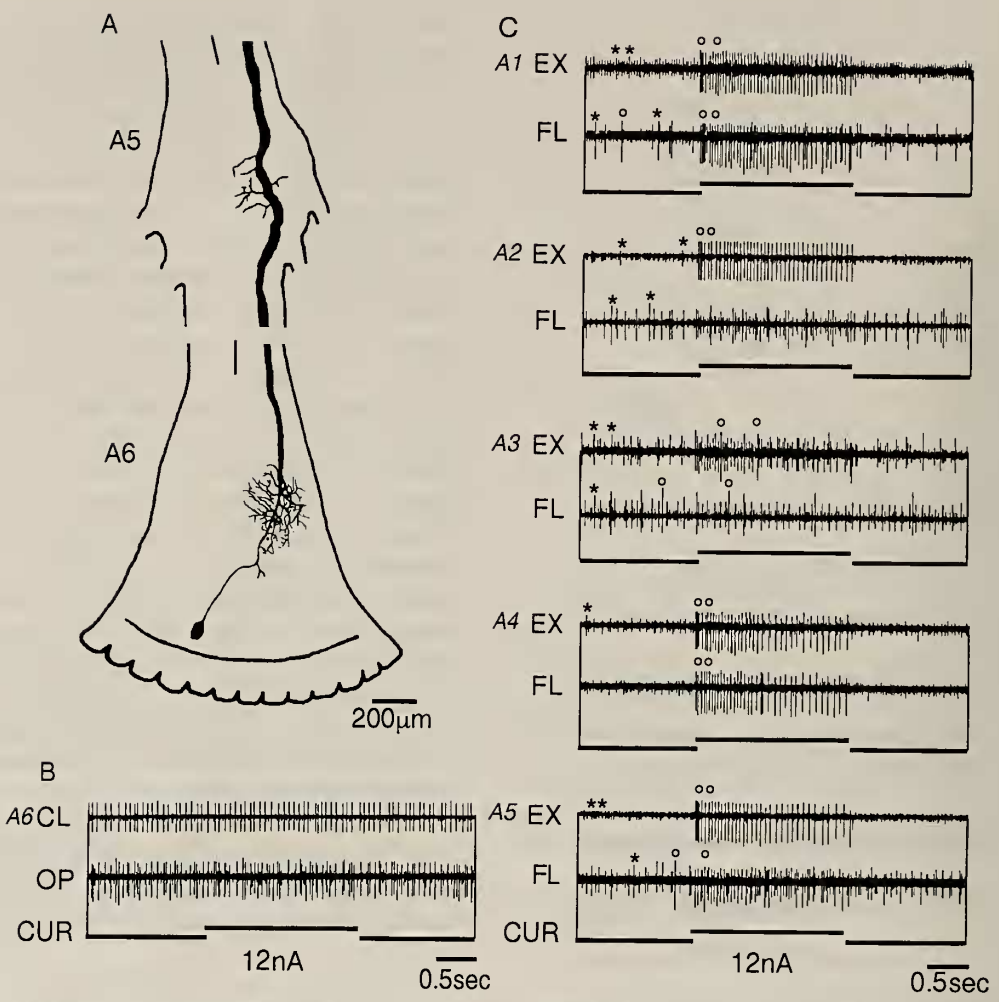

Fig. 4. Output effect of NE-1. A. Morphology of NE-1 in the 5th and terminal (A6) abdominal ganglion. The projection of the axonal branches (A5) was in the ipsilateral neuropil only. B. Output effect of NE-1 upon both the closer (1st trace) and opener (2nd trace) motor neurons. C. Output effect of NE-1 upon both the extensor and flexor motor neurons in the abdominal (A1-A5) ganglia.

most posterior secondary neurite crossing the midline (A6 in Fig. 6). Neurobiotin staining revealed that the ascending axon of VE-1 projected into at least the 2nd to 3rd abdominal connective. In each anterior abdominal ganglion (A5, 4 or 3), several small branches extended from the axon within the unilateral half of the neuropil (Fig. 6A). The extent and number of axonal branches in each ganglion were similar. The anterior, medial and posterior branches projected both medially and laterally. These axonal branches were usually extended within the ventral half of the neuropil (Fig. 6B, C). One of the physiological characteristics of VE-1 was variable output effect upon the uropod motor neurons [21]. In this study, VE-1 was impaled 19 times but their effects upon the uropod motor neurons were inconsistent: 3 interneurons activated both the closer and opener motor neurons while 13 interneurons activated the closer motor neurons and inhibited the antagonistic opener motor neurons. The remaining 3 interneurons had no effect upon the uropod motoneurons. The output effect upon the abdominal postural motor neurons was, by contrast, consistent in almost all preparations. On 17 occasions, this interneuron decreased spike frequency of the extensor inhibitor with the increase in the activity of the extensor excitors (No. 3/4) in all the anterior ganglia (Fig. 7A). At the same time, tonic spikes of the flexor excitors (No. 3/4) in all the anterior ganglia were inhibited with the increase in the spikes of the flexor inhibitor. Another 2 VE-1 had no effect upon the postural motor neurons in any anterior ganglia. In many cases, VE-1 also affected the postural motor neurons on the contralateral side. The output effect was the same as that upon the ipsilateral postural motor neurons and elicited reciprocal extension-like pattern from the 1st to 5th abdominal ganglion (Fig. 7B).

Interneurons with flexion effect

Four identified ascending interneurons, RC-4, RO-2, RO-3 and RO-4 [21], elicited abdominal flexion-like motor pattern. For example, figure 8A showed the morphology of RO-2 in the terminal abdominal ganglion. Physiologically, RO-2 showed antifacilitation in responses to repetitive electrical stimulation of the afferents. Spikes followed only the

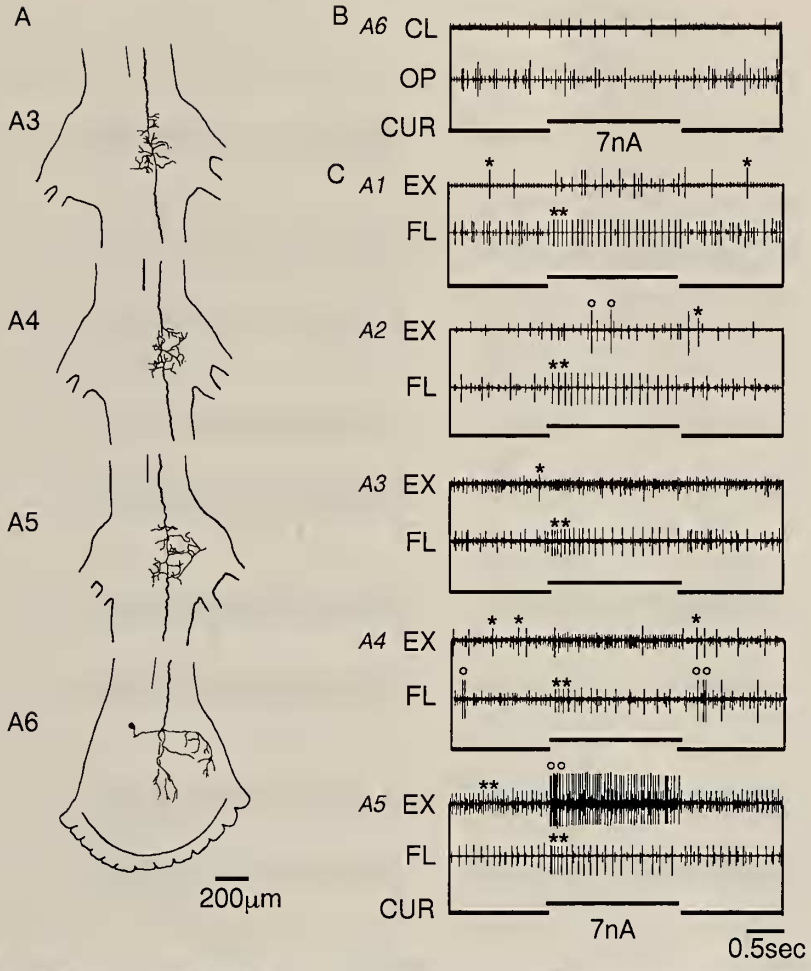

FIG. 5. Output effect of RC-2. A. Morphology of RC-2 from the 3th to terminal (A3-A6) abdominal ganglion. The projection of the axonal branches in each abdominal ganglion (A1-A5) was in the ipsilateral neuropil only. B. Output effect of RC-2 upon both the closer and opener motor neurons. C. Output effect of RC-2 upon both the extensor and flexor motor neurons in the anterior abdominal (A1-A5) ganglia.

1st stimulus and then continuously depressed after the 2nd stimulus (Fig. 8B). When the depolarizing current was injected into RO-2, tonic spikes of the opener motor neurons increased while those of the closer motor neurons decreased (not shown). At the same time, this interneuron increased the spike frequency of extensor inhibitor (No. 5) and also activated that of the flexor excitors (No. 3/4 and 6) in all the anterior abdominal ganglia (Fig. 8C). Another 3 interneurons, i.e., RC-4, RO-3, RO-4, had similar output effects upon the abdominal postural motor neurons, though the effect of RC-4 upon the uropod motor neurons was opposite from other interneurons (Table 1).

DISCUSSION

Output effect of ascending interneurons upon abdominal postural system

This study has demonstrated that many identified ascending interneurons originating in the terminal abdominal ganglion of the crayfish [21] have premotor effects upon the abdominal postural motor neurons in the anterior abdominal ganglia. Each interneuron controlled the homologous postural motor neurons from the 1st to 5th abdominal ganglia simultaneously in the same way (Table 1). For example, if the extensor excitors in the 5th abdominal ganglion were excited by a particular interneuron, the activity of extensor excitors in the remaining abdominal ganglia (A1-A4) were also increased. These physiological results suggested that the axons of ascending interneurons ran through the abdominal connective and projected into thoracic or more anterior ganglia. Neurobiotin staining could reveal the intersegmental structure of ascending interneurons, since this dye spreaded rapidly for a long distance (about 3 cm). The ascending interneurons extended several axonal branches with similar projection in each anterior ganglion (e.g. Fig. 6), though we could not trace the whole structure of interneurons.

The patterns of output effects of ascending interneurons upon the abdominal postural motor neurons were divided into three types. About half of interneurons encountered in this study (7 out of 16 interneurons) co-actively excited both

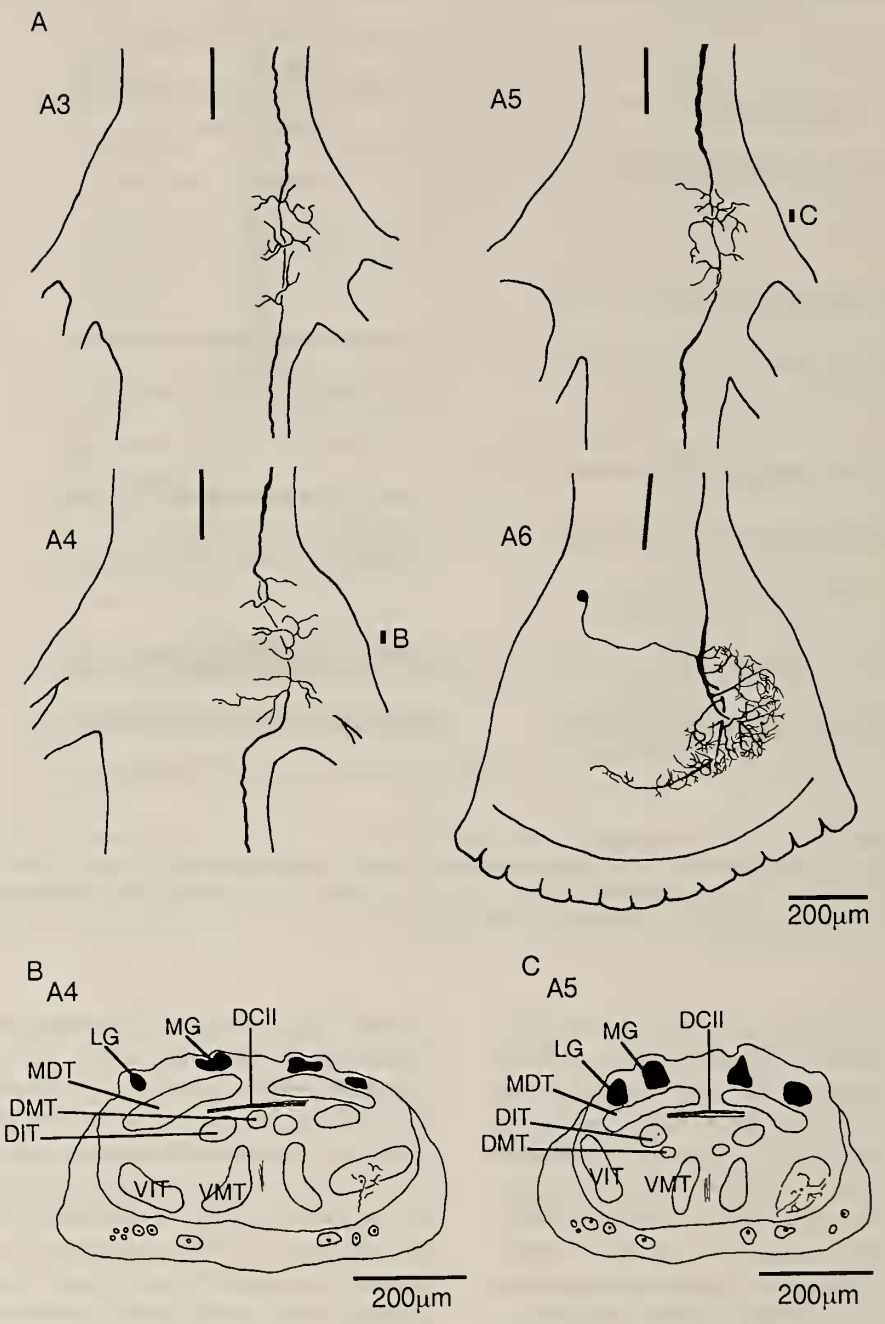

FIG. 6. Morphology of VE-1. A. Morphology of ascending interneuron VE-1 in the 3rd (A3), 4th (A4), 5th (A5), and terminal (A6) ganglion. The projections of the axonal branches in the anterior abdominal ganglia (A3-A5) were in the ipsilateral neuropil only. B, C. Drawing of sections (20 μm) of the 4th and 5th abdominal ganglion. Transverse sections arranged from the homologous part of each ganglion. The axon of VE-1 in each ganglion ran through ventral intermediate tract and project axonal branches both medially and laterally. DC II,dorsal commissure II; DIT, dorsal intermediate tract; DMT, dorsal medial tract; LG, lateral giant; MDT, medial dorsal tract; MG, medial giant; VIT, ventral intermediate tract; VMT, ventral medial tract.

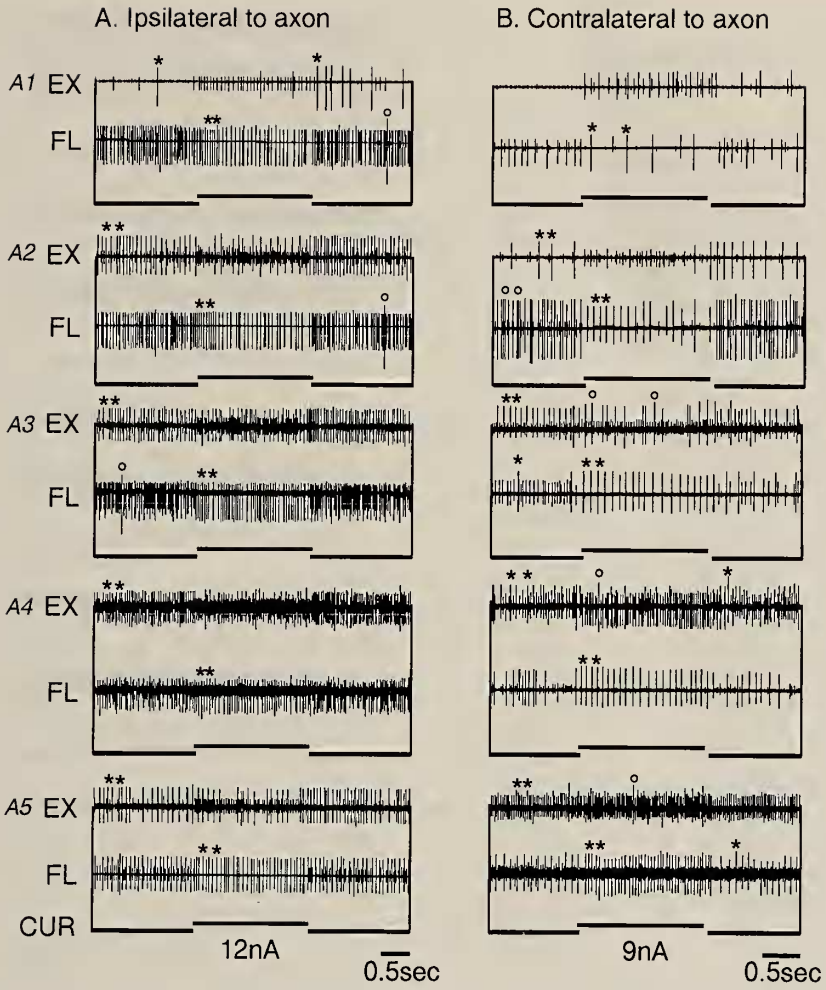

FIG. 7. Output effect of VE-1 upon abdominal postural motor neurons. A. Activity change of extensor (1st trace in each ganglion) and flexor (2nd trace in each ganglion) motor neurons on the side ipsilateral to the axonal branches of VE-1. The response of motor neurons from the 1st to 5th abdominal ganglion was drawn succeedingly. B. The response of postural motor neurons on the opposite side. On the flexor motor neurons in the 3rd abdominal ganglion (2nd trace in A3), the largest tonic flexor excitor (No. 6) spiked spontaneously and the current injection decreased the spike (No. 6) and increased the flexor inhibitor (No. 5).

the extensor and flexor excitors. Four interneurons elicited reciprocally extension-like motor pattern while another 4 interneurons elicited reversed flexion-like motor pattern. In both the crayfish and lobster, abdominal positioning interneurons have been known to produce abdominal movement [5, 6, 13, 14]. Many of them had somata in the anterior abdominal ganglia (A2-A5) and had ascending and/or descending axons through the abdominal connective. Parts of them were originated from the terminal abdominal ganglion and some of them might be similar to the interneurons described in this study [5]. In the previous works, abdominal positioning interneurons were categorized as one of either FPIs (flexion producing interneurons), EPIs (extension producing interneurons) or inhibitory interneurons [e.g. 6]. By contrast, no interneurons in this study inhibited both the extensor and flexor excitors, but many interneurons coactively excited both the antagonistic motor neurons.

Multiple function of ascending interneurons

Ascending interneurons in the terminal ganglion received excitatory input directly from the mechanosensory afferents innervating hairs on the surface of the tailfan [23] and/or from the proprioceptive afferents innervating the chordotonal organ of the uropod [28]. They, in turn, propagated encoded sensory signals into anterior segments and affected abdominal postural motor neurons in all the anterior (A1-A5) abdominal ganglia [this study]. At the same time, many interneurons also affected the uropod motor neurons in a various fashion [21]. Some interneurons further recruited the unidentified motor neurons of swimmerets (1st root) that were homologous appendages with uropods, though rhythmic period of power- and return-stroke was not affected [Aonuma, unpublished data]. The ascending interneurons, therefore, acted as multifunctional units that controlled the different motor systems simultaneously.

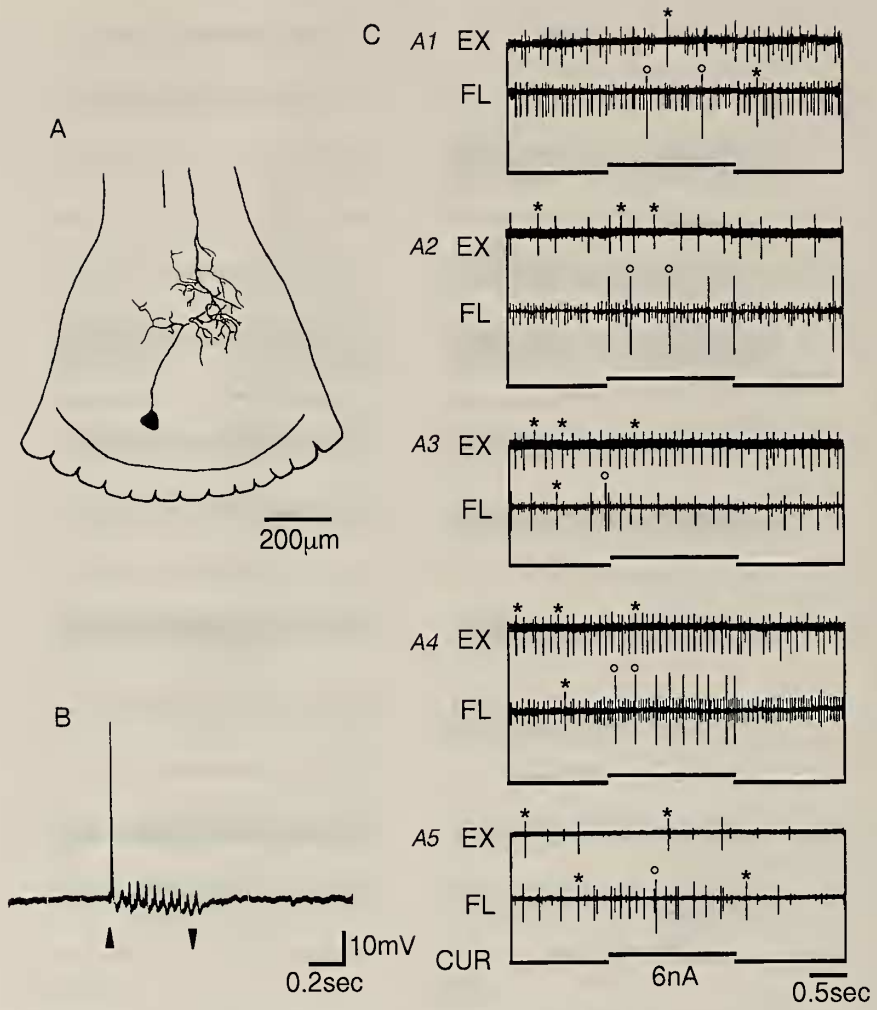

Fig. 8. Output effect of RO-2. A. Morphology of RO-2. B. Repetitive electrical stimulation (indicated by arrowheads) of the 2nd root afferent (20 Hz) elicited antifacilitation in RO-2. C. Output effect of RO-2 upon both the extensor and flexor motor neurons in the abdominal (A1-A5) ganglia.

There was, however, no close correlation between the output effect upon the uropod motor neurons and that upon the abdominal postural motor neurons (Table 1). Interneurons that elicited reciprocally closing pattern of the uropod produced either extension-like or flexion-like motor pattern of the abdomen. Furthermore, interneurons that activated both the extensor and flexor excitors had different effect upon the uropod motor neurons. CA-1 excited both the closer and opener motor neurons while CI-1 inhibited both motor neurons. Interneurons of RC group (RC-3, -6, -8) produced closing pattern of the uropod, while RO-5 produced reversed opening pattern. Only interneurons with flexion effect tended to elicit the reciprocally opening pattern of the uropod (3 out of 4 interneurons). This variety of combination of output to uropod and abdominal postural system in ascending interneurons would partly be derived from the complexity of movement of the abdominal segments. During equilibrium reactions [41], leg reflexes [27], avoidance reaction [26], escape swimming [39], backward walking [12] and defensive reaction [29], coordinated movement with a various pattern between abdomen and uropod was performed. The intersegmental coordination between uropod and abdomen was also essential for posture and locomotion of animals. For example, the flexion producing interneurons (e.g. RO-2, -3 and -4) would be related to the LG mediated tail-flip that was initiated by the rapid flexion of the abdomen [39]. On the other hand, RC-2 and RC-7 had potential to mediate avoidance "dart" response that consisted of immediate closing of uropods followed by the forward walking with abdominal extension [26]. These interneurons produced a train of spikes in response to the repetitive sensory stimulation, elicited the closing pattern of the uropod and produced the abdominal extension (Fig. 5). The physiological result that interneurons with flexion effect failed to respond spikes continuously by the repetitive sensory stimulation of the tailfan as a result of antifacilitation (Table 1) might be consistent with the behavioural observation that the mechanical stimulation of the tailfan preferably elicited the "dart" response (Fig. 2A). Furthermore, slow postural movement of the abdomen was usually accompanied with the activation of both the extensor and flexor muscles [30]. The interneurons such as CA-1, RC-3 and RC-6 would contribute

to increase the tonus of the postural muscles, since they co-actively excited both the extensor and flexor excitors and/or inhibited the inhibitors. Thus, each ascending interneuron will contribute to one or more specific behavioural act(s) and the coordinated sequence of movement between uropod and abdominal posture will be completed by the activation of these ascending interneurons as multisegmental integrators. A particular behavioural act might be triggered through selective activation and certain central interaction between these ascending interneurons.

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